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Preliminary study on antifertility activity of *Enicostemma axillare* leaves and *Urena lobata* root used in Indian traditional folk medicine

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ABSTRACT

Objective: To evaluate the possible antifertility activity of *Enicostemma axillare* (*E. axillare*) leaves and *Urena lobata* (*U. lobata*) root in adult male Wistar albino rats. **Methods:** Six groups of rats were treated with ethanolic (70%v/v) extracts of *E. axillare* (375 and 750 mg/kg body weight) and *U. lobata* root (300 and 600 mg/kg body weight) once daily for 55 days. Control groups received the distilled water and vehicle. All the treated rats had corresponding recovery groups. At the end of each treatment periods, animals were killed and organ weights, sperm characteristics, testicular and epididymal biochemicals as well as testicular enzymes were assessed. **Results:** The *E. axillare* and *U. lobata* at tested doses did not decrease body weight, whereas the weight of testes, epididymides and seminal vesicles were significantly ($P<0.01$) reduced. Significantly ($P<0.01$) more reductions in the sperm motility, viability and counts, epididymal and testicular protein contents were noted in the rats treated with higher dose of both the plants. Both the plants at the higher dose caused a marked increase ($P<0.01$) in sperm morphological abnormalities, testicular cholesterol and ascorbic acid contents were remarkably increased ($P<0.01$), while, the activities of testicular glucose-6-phosphate dehydrogenase (G-6-PDH) and Δ^5-3 β -hydroxy steroid dehydrogenase (Δ^5-3 β -HSD) were significantly reduced ($P<0.01$). However, reversal of these changes occurred after 55 days of treatment withdrawal. **Conclusions:** This study suggests that the *E. axillare* leaves and *U. lobata* root reversibly inhibited spermatogenesis and steroidogenesis indicating reversible antifertility activity which could partially support the traditional of these plants as male contraceptives.

1. Introduction

Population control remains one of humankind's greatest challenges in the 21st century. The population burden continues to tax our environment and contribute to morbidity and mortality globally. It is the hope of researchers in the field of male contraceptive development that approaches to blocking sperm production and function will bring the dream of the "male pill" to fruition and significantly impact the health and future of the planet[1]. The folklore information and ancient literature about the plants and herbs can help the antifertility program[2]. Research on Indian plants with male antifertility activity has been exhaustively reviewed recently[2-4]. However, the search for effective, safe and orally active plant products are yet to be needed for fertility regulation.

Enicostemma axillare (Lam.) Raynal. (Syn. *Enicostema littorale Blume*) (*E. axillare*), belongs to the family Gentianaceae, (Family Gentianaceae) is a perennial herb found throughout India and common in coastal areas[5]. The plant is used in folk medicine to treat diabetes mellitus, rheumatism, abdominal ulcers, hernia, swelling, itching and insect poisoning[6]. In recent years, this plant is reported to possess anti inflammatory[7], hypolipidemic and antioxidant antidiabetic[8], hepatoprotective[9], antinociceptive[10] and antimicrobial properties[11].

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Urena lobata Linn (Family: Malvaceae) (*U. lobata*) is an annual shrub of 60-250 cm or more height and distributed in hotter parts of India[12]. In traditional medicine, this plant is being used to cure diarrhea, colic, skin diseases, boils, pneumonia, rheumatism, cough, and diabetes in India, Indonesia, Malaysia, Philippines, Cuba, and Nepal[13-16]. Roots have been reported to possess a broad spectrum of antibacterial activity[13,17]. In recent years, the root of this plant has been shown to have anti-diabetic[18] and immunomodulatory[19] effects. Imperatorin, a furocoumarin was isolated from the roots[20].

In addition, the information collected from local traditional practioners in different places of southern India revealed that the *E. axillare* leaves and *U. lobata* root are being taken orally to develop sterility in man. However, the proper scientific researches on antifertility effects these two plants have not been carried out before to verify the traditional claims. Based on this background, therefore, the present study was undertaken to evaluate the possible antifertility activity at various doses of 70% ethanol extract *E. axillare* leaves and *U. lobata* root in male albino rats.

2. Materials and methods

2.1. Plant materials and preparation of extracts

The leaves of *E. axillare* and roots of *U. lobata* were collected from Sillathur village of Thanjavur District, Tamilnadu, India, in the year of 2006. The plants specimen was authenticated by Dr. P. Jayaraman, M.Sc., Ph.D, Plant Anatomy Research Centre (PARC), Chennai Tamil Nadu, India. A voucher specimen (*E. axillare*-PARC/2006/378 and *U. lobata* -PARC/2006/383) have been deposited in the herbarium of the same department. The pars of the plants were separately dried in shade, pulverized by a mechanical grinder and passed through 40-mesh sieve and stored in airtight container for further use.

The powdered dry parts of the selected plants about 500 g were individually extracted successively with 70% v/v ethanol at 68 ℃ in Sohxlet apparatus. The extracts were collected in 5 L individual conical flasks, filtered, and the solvent was evaporated to dryness under reduced pressure in an Eyela Rotary Evaporator (Japan) at 40-45 ℃ and were stored in a vacuum desiccators. The yields (% w/w) of the prepared extracts were found to be 9.6% and 12.4% for *E. axillare* leaves and *U. lobata* root extracts, respectively, with respect to dried powder. The extracts were dissolved individually in 1% Tween80 solution and were used for experimental purpose.

2.2. Animals used

Adult Wistar strain male and female albino rats, *Rattus norvegicus* (90 days old), weighing 150-200 g, procured from Animal house, were housed in groups of five per cage made of polypropylene (8″×12″×8″) with metal grill tops. The animals were maintained with standard pellet feed (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*. All experimental procedures described were reviewed and approved by the University Animal Ethical Committee[21].

2.3. Acute oral toxicity study

The ethanolic extracts of *E. axillare* leaves and *U. lobata* root were administered as a single dose using a gastric intubation tube after fasting for 3 to 4 h. The substance is tested using a stepwise procedure, each step using three animals of a single sex (females). Since there was no information on the substance to be tested (*i.e.* extracts), starting dose was 2 000 mg/kg body weight up to 5 000 mg/kg body weight. Animals were observed initially after dosing at least once during the first 30 min, periodically during the first 24 h. In all cases death was observed within first 24 h. Attention was also given to observations of tremors and convulsions[22].

2.4. Design of experiment

Sixty healthy male albino rats were selected and divided into six groups containing ten rats each and treated as follows:

Group-1 received distilled water (10 mL/kg. b.w) as normal control; Group-2 received 1% Tween 80 dissolved in distilled water (10 mL/kg b.w) as vehicle control; Groups-3 and 4 received ethanolic extract of *E. axillare* leaves at the doses of 375 and 750 mg/kg body weight, respectively, suspended in 1% Tween 80; Groups-5 and 6 received and *U. lobata* root at the doses of 250 and 500 mg/kg b.w respectively, suspended in 1% Tween 80.

The vehicles and plant drugs were administered intragastric (*i.g.*) route on consecutive days for 55 days. At the end of the experimental period, five animals from both control and experimental groups were given anesthesia under mild sodium pentobarbital 24 h after the last dose and 18 h after fasting. Blood was also obtained by cardiac puncture from these animals for the analysis of hematological profiles. The testis, cauda epididymal ducts and seminal vesicles were dissected out, trimmed off from adherent fats and weighed and recorded to the nearest milligram on a digital balance. Sperm from cauda epididymal ducts were released in phosphate buffer solution (pH-9.0) media and used for spermatological studies. The testes were used for biochemical estimation. The remaining five rats from groups (3-6) were left for recovery studies over a period of next 55 days (from 56th day to 110th day). All spermatological parameters were repeated in order to ascertain the nature of action of extract, *i.e.* reversible or irreversible.

The body weights of the animals were recorded, prior to and after treatment and recovery. Testis, epididymis, seminal vesicles and ventral prostate gland were weighted to the nearest milligrams.

2.5. Spermatological studies

The cauda epididymal duct on one side was exposed and incised. The connective tissue capsule around the cauda epididymidis was teased out and the epididymal duct was uncoiled. The semen that oozed into the cavity block was quickly sucked into a capillary tube up to 0.05 μL mark and transferred to an Eppendorf tube. It was diluted 200 (0.05 μL of sperm with 99.95 μL of PBS) times in physiological saline. After thorough mixing, the sperm

suspension was used for analysis of motility[23].

A drop of dilute semen was transferred to an Eppendorf tube containing one drop of 10% nigrosine and one drop of 1% eosin then the sperm viability test was done by the method as described in the WHO Laboratory Manual[24]. Sperm morphology was observed adopting Papanicolaou staining. The staining solutions were prepared according to the method of Raphael[25]. Sperm counts were made according to the method described by Gopalakrishnan[26].

2.6. Biochemical estimations

2.6.1. Estimation of cholesterol content

Testis tissue was carefully homogenized in Potter Elvehjem homogenizer using chloroform: ethanol mixture (2:1) and non-polar part was extracted out. The optical density was determined in spectrophotometer at 620 nm against blank (chloroform) and total cholesterol content was estimated according to the method of Sperry and Webb[27].

2.6.2. Estimation of ascorbic acid content

Testis tissue was homogenized in Potter Elvehjem homogenizer using 45 μL ice cold 5% meta-phosphoric acid and centrifuged for 20 min at 3 500 *g*. Then, 30 μL supernatant, 15 μL acetate buffer, and 15 μL of 2, 6-dichlorophenol-indophenol sodium (0.1 mg/mL) were mixed and optical density was measured against blank (distilled water) at 520 nm. Standard curve was drawn against known concentrations of ascorbic acid content[28].

2.6.3. Estimation of Δ^5-3 β -hydroxysteroid dehydrogenase (Δ^5-3 β -HSD)

Testis were homogenized in 0.1 M phosphate buffer (pH 7.4) and centrifuged at 10 000 *g* for 10 min at 0 ℃. Nicotinamide adenine dinucleotide (0.2 mL) and 0.1 mL of dihydroxyepiandrosterone were added to supernatant and mixed well. This solution was kept in shaking incubator at 35 ℃ for 90 min, acidified with 0.1 mL 3 M acetate buffer (pH 5.0) and extracted with 10 mL of ethyl acetate and evaporated. Residue was dissolved in 2 mL of ethanol and optical density was measured at 240 nm against blank (ethanol). The activity was estimated by the method of Rabin *et al*[29]. The protein content of tissue was determined and specific activity was expressed per mg of protein.

2.6.4. Estimation of glucose-6-phosphate dehydrogenase (G-6-PDH)

Testis were homogenized and centrifuged at 1 000 *g* (5 min) and 10 000×*g* (10 min) at 0 ℃. Tris-HCl buffer (0.025 mL; pH 8.3; 0.5 M), 0.01 mL of 20 mM of nicotinamide adenine dinucleotide phosphate, 0.02 mL of supernatant, and 0.025 mL of glass distilled water was added and mixed well and optical density was measured at 340 nm against blank (distilled water). The activity of G-6-PDH was estimated by the method of Lohr and Waller[30].

Protein was estimated with Folin's phenol reagent and the activities of enzymes were expressed in unit per mg of protein as described by Lowry *et al*[31]. Fructose content in seminal vesicle was measured as described in WHO Laboratory Manual[32].

2.7. Statistical analysis

Results are expressed as mean±SEM. Data obtained were statistically analysed by using Graphpad Prism, version 4.03 for Windows (Graph Pad Software, San Diego, California, USA). Results were compared using one-factor analysis of variance (ANOVA) with Dunnett's *post-hoc* test. Values were considered significant at $P<0.05$ or less. In many cases results were calculated as percentage of relevant control values to make understanding of the results easier.

3. Results

3.1. Acute oral toxicity

The LD_{50} Cut off value was found to be 3 750 and 2 500 mg/kg body weight for ethanolic (70%v/v) extracts of *E. axillare* leaves and *U. lobata* root, respectively.

3.2. Body weight and the weight of reproductive organs

When compared with vehicle control group, there were no significant differences in initial and final body weights between rats treated with *E. axillare* leaves and *U. lobata* root at both tested doses (Table 1). On the other hand, the weight of the testes, epididymides, seminal vesicle, ventral prostate and vas deferens however, were found to be significantly ($P<0.05$) decreased in treated male rats when compared with the weights of the same organs obtained from control rats (Table 2). However, these organs weights recovered gradually to control levels by 55 days after cessation of treatment (data not shown).

3.3. Effects on sperm morphology and viability

Results recorded in Table 3 showed that percentages of living sperms decreased significantly in rats treated with the *E. axillare* leaves (74.7% by low dose and 66.3% by high dose) and *U. lobata* root (62.5% by low dose and 43.8% by high dose) when compared with vehicle control (92.0%). At the same time, in the vehicle control fed rats, 90.6% of spermatozoa possess normal morphology whereas, *E. axillare* leaves extract-treated rats showed about 76.6% and 73.4% of normal sperm morphology at the doses of 375 and 750 mg/kg body weight, respectively, and *U. lobata* root extract-treated rats showed the corresponding percentage about 72.8% and 46.3% at the doses of 300 and 600 mg/kg body weight, respectively (Table 3). The remaining sperms showed abnormalities of different types.

3.4. Effect on sperm count and sperm motility

Cauda epididymal sperm count was decreased significantly after the treatment with *E. axillare* leaves and *U. lobata* root extracts for 55 days in comparison to the control group. Further, rats treated with *U. lobata* root at high dose (600 mg/kg body weight) showed more reduction in sperm count and motility than *E. axillare* leaves treated rats when compared with vehicle control group (Tables 3 and 4).

3.5. Effect on epididymal protein and fructose content in the seminal vesicle

The *E. axillare* leaves and *U. lobata* root at two different doses decreased the seminal vesicular fructose content by 12.24% & 22.44% and 14.28% & 24.48%, respectively, when compared to vehicle control group (Table 4). In case of epididymal protein content, the *U. lobata* root at the doses of 300 and 600 mg/kg body weight showed more of reduction (10.60% and 14.46%, respectively) than *E. axillare* leaves (6.00% by low dose and 8.82% by high dose) when compared with vehicle control group. However, levels of seminal vesicular fructose and epididymal protein in the rats were recovered gradually to control levels by 55 days after cessation of treatment (Table 4).

3.6. Effect on testicular cholesterol, ascorbic acid and protein content

When compared to vehicle control group, significantly ($P<0.05$) a dose-dependent elevation of cholesterol and ascorbic acid contents were noted in rats treated with both the plants (Table 5). Moreover, *U. lobata* root at high dose (600 mg/kg body weight) showed more elevation of cholesterol and ascorbic acid content by 32.99% and 34.33%, respectively, than *E. axillare* leaves (21.83% and 16.50%, respectively, at high dose) when compared with vehicle control group (Table 5). At the same time, treatment with both the plants resulted in reduction of protein content of testis. This reduction was more significant ($P<0.01$) in *U. lobata* root (19.22% by low dose and 24.08% by high dose) treated rats than *E. axillare* leaves-treated groups (9.07%

Table 1

Effect of ethanolic extracts of *E. axillare* leaves and *U. lobata* root on body weight of rats after 55 days of treatment (Mean±SEM, n=5).

Treatment design	Dose (mg/kg body weight)	Body weight		
		Initial weight(g)	Weight after treatment (g)	% increase in weight
Group 1 (Normal)	10 mL	181.16±4.63	236.43±6.64	30.50
Group 2 (Vehicle)	10 mL	180.43±3.27	234.47±8.53	29.95
Group 3 (EEEA)	375 mg	182.54±6.17	236.83±4.44	29.74 ^{ns}
Group 4 (EEEA)	750 mg	180.26±3.28	233.62±6.41	29.60 ^{ns}
Group 5 (EEUL)	300 mg	181.14±4.63	232.65±3.47	28.43 ^{ns}
Group 6 (EEUL)	600 mg	183.17±2.78	236.45±4.62	29.08 ^{ns}

ns= Non-significantly different from vehicle control.
EEEA and EEUL= ethanolic extracts of *E. axillare* leaves and *U. lobata* root, respectively.

Table 2

Data on the weight of reproductive organs of male rats after treated with ethanolic extracts of *E. axillare* leaves and *U. lobata* root (Mean±SEM, n=5).

Treatment design	Dose (mg/kg body weight)	Weight of reproductive organs				
		Testis (g)	Caput epididimidis (mg)	Cauda epididimidis (mg)	Seminal vesicles (mg)	Ventral prostate (mg)
Group 1 (Normal)	10 mL	2.81±0.08	348.86±5.74	237.46±7.76	286.52±15.66	135.14±3.16
Group 2 (Vehicle)	10 mL	2.82±0.07	347.65±8.76	235.63±8.46	287.46±13.57	134.11±4.23
Group 3 (EEEA)	375 mg	2.62±0.04* (-7.09)	328.46±2.73* (-5.51)	215.56±3.48* (-8.51)	263.24±4.37* (-8.42)	121.32±2.14 *(-9.53)
Group 4 (EEEA)	750 mg	2.43±0.03** (-13.82)	311.36±3.64** (-10.43)	202.42±2.53** (-14.09)	252.17±2.46** (-12.27)	115.36±1.86** (-13.98)
Group 5 (EEUL)	300 mg	2.40±0.01** (-14.87)	323.57±1.44** (-6.92)	214.26±4.31 *(-9.06)	259.34±1.48* (-9.78)	120.22±1.67* (-10.35)
Group 6 (EEUL)	600 mg	2.24±0.06** (-20.56)	316.41±2.32** (-8.98)	196.34±3.76** (-16.67)	247.22±2.42** (-13.99)	108.14±2.36** (-19.36)

*$P<0.05$, **$P<0.01$ significantly different from vehicle control.
EEEA and EEUL= ethanolic extracts of *E. axillare* leaves and *U. lobata* root, respectively.

Table 3

Effect of ethanolic extracts of *E. axillare* leaves and *U. lobata* root on perm viability, morphology and sperm count after 55 days of treatment and after 56-110 days of treatment withdrawal (Mean±SEM, n=5).

Treatment design	Dose (mg/kg body weight)	Sperm viability (%)		Sperm morphology				Sperm count (×10^{6}sperm/mL)	
				Normal sperm (%)		Abnormal sperm (%)			
		After treatment	Recovery	After treatment	Recovery	After treatment	Recovery	After treatment	Recovery
Group 1 (Normal)	10 mL	89.4±1.6	90.2±1.3	91.3±2.7	91.4±2.3	8.7±1.6	8.2±1.7	65.4±2.3	66.3±1.2
Group 2 (Vehicle)	10 mL	88.2±1.4	89.3±1.5	90.6±3.2	92.3±1.2	9.3±1.4	7.6±2.4	64.8±3.6	66.7±2.4
Group 3 (EEEA)	375 mg	74.7±2.1**	89.2±0.4	76.6±1.4*	91.2±1.3	21.3±1.8*	9.2±1.3	53.2±1.4**	64.5±1.7
Group 4 (EEEA)	750 mg	66.3±1.5**	87.6±1.6	73.4±2.6**	89.3±2.4	31.1±3.3**	10.1±1.7	46.6±2.5**	62.8±2.3
Group 5 (EEUL)	300 mg	62.5±2.2**	86.4±0.8	72.8±4.3**	88.4±1.6	36.6±2.4**	9.0±1.6	52.7±2.6**	63.4±2.8
Group 6 (EEUL)	600 mg	43.8±1.6**	83.7±1.2	46.3±2.4**	87.5±2.2	63.2±1.8**	10.3±0.4	43.2±2.4**	61.7±3.2

*$P<0.05$, **$P<0.01$ significantly different from vehicle control.
EEEA and EEUL= ethanolic extracts of *E. axillare* leaves and *U. lobata* root, respectively.

by low dose and 17.02% by high dose) as compared to that of vehicle control (Table 5). However, by 55 days of treatment withdrawal, the values recovered to control levels (Table 5).

3.7. Effect on G-6-PDH and Δ^5-3 β -HSD activities

The extracts of both the plants were found to exhibit a dose dependent decrease in testicular G-6-PDH activity when compared with control. After 55 days of treatment, the percent inhibition of G-6-PDH activity at two different doses was 6.35% and 18.49% for *E. axillare* leaves (375 and 750 mg/kg body weight) and 16.42% and 34.74% for *U. lobata* root (300 and 600 mg/kg body weight), respectively (Table 6). Similarly, the *E. axillare* leaves and *U. lobata* root at two different doses inhibited the testicular Δ^5-3 β -HSD activity by 18.74% & 24.66% and 21.76% & 33.01%, respectively, when compared to vehicle control group. However, by 55 days of treatment withdrawal, the values recovered to control levels (Table 6).

Table 4

Effect of ethanolic extracts of *E. axillare* leaves and *U. lobata* root on motility and type of movement of sperm, epididymal protein and content of fructose in seminal vesicular fructose content after 55 days of treatment and after 56-110 days of treatment withdrawal (Mean±SEM, *n*=5).

Treatment design	Types of motility		Duration of motility (min)		Fructose in seminal vesicle (mg/g)		Protein in epididymis (mg/g)	
	After treatment	Recovery	After treatment	Recovery	After treatment	Recovery	After treatment	Recovery
Group 1 (Normal, 10 mL/kg body weight)	Rapid progressive	Rapid progressive	105±2	104±3	4.80±0.23	4.80±0.02	218.53±4.67	216.43±2.12
Group 2 (Vehicle, 10 mL/kg body weight)	Rapid progressive	Rapid progressive	103±3	103±5	4.90±0.22	4.80±0.04	215.38±5.86	214.14±3.35
Group 3 (EEEA 375 mg/kg body weight)	Mild progressive	Rapid progressive	78±4*	100±3	4.30±0.13* (-12.24)	4.80±0.03	202.45±3.16* (-6.0)	212.45±2.24
Group 4 (EEEA 750 mg/kg body weight)	Sluggish	Progressive	56±3**	99±4	3.80±0.14** (-22.44)	4.70±0.14	196.37±2.64** (-8.82)	214.12±0.76
Group 5 (EEUL 300 mg/kg body weight)	Mild progressive	Progressive	62±3**	101±2	4.20±0.12** (-14.28)	4.80±0.01	192.54±1.35** (-10.60)	211.63±1.17
Group 6 (EEUL 600 mg/kg body weight)	Sluggish	Progressive	45±2**	99±3	3.70±0.13** (-24.48)	4.70±0.06	184.23±1.85** (-14.46)	210.56±4.32

*P<0.05, **P<0.01 significantly different from vehicle control.
EEEA and EEUL= ethanolic extracts of *E. axillare* leaves and *U. lobata* root, respectively.

Table 5

Effect of ethanolic extracts of *E. axillare* leaves and *U. lobata* root on testicular cholesterol, ascorbic acid and protein content in rats after 55 days of treatment and after 56-110 days of treatment withdrawal (Mean±SEM, *n*=5).

Treatment design	Cholesterol(μ g/mg of tissue)		Ascorbic acid(μ g/mg of tissue)		Protein(mg/g of tissue)	
	After treatment	Recovery	After treatment	Recovery	After treatment	Recovery
Group 1 (Normal, 10 mL/kg body weight)	87.26±2.34	88. 60±1.26	138.61±2.17	139.42±1.24	186.30±2.40	191.50±1.40
Group 2 (Vehicle, 10 mL/kg body weight)	88.43±2.42	89.12±1.38	137.83±1.36	138.34±1.53	188.50±1.70	190.60±1.80
Group 3 (EEEA 375 mg/kg body weight)	98.81±1.34* (+11.73)	87.35±1.14	148.85±2.64* (+7.99)	136.42±0.48	171.40±1.20** (-9.07)	188.80±0.60
Group 4 (EEEA 750 mg/kg body weight)	107.74±2.82** (+21.83)	87.74±1.32	160.58±1.21** (+16.50)	137.12±0.14	156.4 0± 2.30** (-17.02)	188.60±0.30
Group 5 (EEUL 300 mg/kg body weight)	106.23±1.16** (+20.12)	87.56±1.48	167.44±2.28** (+21.48)	136.86±1.32	152.20±1.40** (-19.22)	188.90±0.50
Group 6 (EEUL 600 mg/kg body weight)	117.61±2.23** (+32.99)	87.47±0.43	185.16±2.24** (+34.33)	136.54±0.36	143.10±1.60** (-24.08)	187.70±0.20

*P<0.05, **P<0.01 significantly different from vehicle control.
EEEA and EEUL= ethanolic extracts of *E. axillare* leaves and *U. lobata* root, respectively.

Table 6

Effect of ethanolic extracts of *E. axillare* leaves and U. lobata root on activities of Δ^5-3 β -HSD and G-6-PDH in testis of rats after 55 days of treatment and after 56-110 days of treatment withdrawal (Mean±SEM, *n*=5).

Treatment design	Specific activity of Δ^5-3 β -HSD(U /mg of protein)		Specific activity of G-6-PDH(U /mg of protein)	
	After treatment	Recovery	After treatment	Recovery
Group 1 (Normal, 10 mL/kg body weight)	8.52±0.06	7.68±0.26	23.22±0.34	23.47±0.16
Group 2 (Vehicle, 10 mL/kg body weight)	8.27±0.12	7.52±0.15	23.14±0.12	23.82±0.31
Group 3 (EEEA 375 mg/kg body weight)	6.72±0.37*(-18.74)	7.36±0.54	21.67±0.54**(-6.35)	23.41±0.04
	6.23±0.26**(-24.66)	7.12±0.14	18.86±0.32**(-18.49)	23.17±0.30
Group 5 (EEUL 300 mg/kg body weight)	6.47±0.24**(-21.76)	7.44± 0.36	19.34±0.33**(-16.42)	23.26±0.13
Group 6 (EEUL 600 mg/kg body weight)	5.54±0.31**(-33.01)	7.41±0.13	15.10±0.24**(-34.74)	23.11±0.14

*P<0.05, **P<0.01 significantly different from vehicle control.
EEEA and EEUL= ethanolic extracts of *E. axillare* leaves and *U. lobata* root, respectively.

4. Discussion

In the present investigation, 10% and 20% of the LD_{50} cut-off value were selected as doses of ethanolic extracts of *E. axillare* leaves or *U. lobata* root. In our study, the results indicated that the body weights of rats treated with *E. axillare* leaves or *U. lobata* root evan at high dose suggesting that the these plants have no side effect or toxicological effect and maintained normal physiology of the animals throughout the experiment.

It is well known that the male accessory reproductive organs play an important role in the sperm maturation, motility and formation of semen[32]. In the present investigation, treatment with *E. axillare* leaves or *U. lobata* root for 55 days resulted in a decrease in weights of testis and cauda epididymis after 55 days of treatment, which could be attributed to a decline in epididymal sperm count and sperm motility inhibition with reduced viability and increased sperm abnormality since the inadequate concentration, sluggishly motile or immotile spermatozoa could not penetrate the cervical mucus and thus failed to fertilize the ova[33, 34]. Moreover, in our study, a low fructose concentration and a reduction in seminal vesicle weight of rats treated with high doses of *E. axillare* leaves or *U. lobata* root would be another cause of reduction in sperm motility since motile sperm consumes fructose after ejaculation[35].

The present study reveals the reduction in the level of protein of testes and epididymis, which might be a causative factor in reducing the weight of reproductive organs, because the growth rate of organ is proportional to its protein content, and may also be another reason for declined sperm count and motility[36-38]. This idea was further strengthened by the accumulation of testicular cholesterol and ascorbic acid contents in rats treated with *E. axillare* leaves or *U. lobata* root for 55 days since these substrates, cholesterol and ascorbic acid are the principal precursors for the formation of androgens in biogenic pathway in the testis[39-41].

The depressed testicular steroidogenic activity and hypofunctioning of the glands was further evident by the diminished values of testicular Δ^5-3 β -HSD and G-6-PDH dehydrogenase activities after treated with both doses of *E. axillare* leaves or *U. lobata* root suggesting both plants might prevent cholesterol conversion into testosterone by impairing activities of these two key regulatory enzymes involved in androgen biogenesis[37,42].

However, after withdrawal of the extract for a period of 55 days, the weight of reproductive organs, sperm count, motility, viability, morphology, testicular biochemicals and enzymes of the extract-treated male rats were similar to those of the vehicle-treated control group, which suggested that the impacts of *E. axillare* leaves and *U. lobata* root extracts on male reproductive functions were reversible.

This study suggests that the *E. axillare* leaves and *U. lobata* root reversibly inhibited spermatogenesis and steroidogenesis indicating reversible antifertility activity which could partially support the traditional of these plants as male contraceptives. Further detailed studies should be carried out using different animal species to establish its antifertility activity, determine the active principles responsible and also understand underlying cellular mechanism of action.

Conflict of interest statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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